Stabilizing role of seed banks and the maintenance of bacterial diversity

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ABSTRACT

Coexisting species often exhibit negative frequency dependence due to mechanisms that promote population growth and persistence when rare. These stabilizing mechanisms maintain diversity through interspecific niche differences, but also through life-history strategies like dormancy that buffer dynamics in fluctuating environments. However, there are few tests demonstrating how seed banks contribute to long-term community dynamics and the maintenance of diversity. Using multi-year, high-frequency bacterial community data from a north temperate lake, we documented patterns consistent with stabilizing coexistence, including differential responses of bacterial taxa to environmental conditions and the presence of a seed bank. By maintaining rare taxa over winter periods, the seed bank contributed to recurrent seasonal dynamics. Last, strong negative frequency dependence in rare, but metabolically active, taxa suggested biotic interactions promoted coexistence. Together, our results provide field-based evidence that niche differences and seed banks stabilize bacterial community dynamics and contribute to the maintenance of microbial diversity in nature.

INTRODUCTION

The maintenance of biodiversity is important for regulating species interactions, stabilizing ecosystem functions, and promoting resilience in response to perturbations. Diversity is maintained by a range of processes including niche differentiation in resource use (Tilman 1982; Gudelj *et al.* 2010; Johnson *et al.* 2012), defensive abilities (Leibold 1996; Thingstad *et al.* 2014; Cadier *et al.* 2019), and abiotic constraints (Holt 2009). These stabilizing niche differences among species contribute to the maintenance of diversity not only by preventing competitive exclusion, but also by allowing populations to recover from low abundances (Chesson 2000; Adler *et al.* 2007). Stabilizing coexistence mechanisms that rely on environmental fluctuations can further increase the number of species in a community (Chesson 1994; Chesson & Huntly 1997; Descamps-Julien & Gonzalez 2005). For example, in seasonal environments, species that are favored at different times of the year may be able to coexist in the community if they can survive through periods of unfavorable environmental conditions (Pake & Venable 1996).

Many species invest in persistence strategies that increase survival and facilitate recovery from rarity. Such strategies can also contribute to stabilization, especially in fluctuating environments where diversity can be maintained by the storage effect (Warner & Chesson 1985; Chesson 2000). The storage effect requires that taxa differ in their responses to environmental conditions, that intraspecific limitation peaks during favorable conditions, and that population growth is buffered in suboptimal environments by a long-lived life stage (Pake & Venable 1996; Cáceres 1997; Angert *et al.* 2009). The storage effect may be particularly important in communities where species experience periods of extremely slow growth (Gray *et al.* 2019) or engage in various forms of dormancy, which are common among plants and animals, but also microorganisms (Lennon & Jones 2011). For example, the accumulation of dormant individuals

62 into a “seed bank” not only reduces the probability of extinction associated with rarity, but it can
63 also facilitate the re-establishment of populations when favorable conditions return via the
64 process of resuscitation. While coexistence mediated by seed banks has been a key hypothesis
65 for the maintenance of microbial diversity (Jones & Lennon 2010; Lennon & Jones 2011;
66 Sorensen & Shade 2020), empirical evidence from temporally fluctuating environments is
67 lacking.

68 Stabilization from niche differences and seed banks should generate negative frequency-
69 dependence (NFD) in population growth. The implication of NFD is that rare populations grow
70 faster than common populations (Chesson 2000; Adler *et al.* 2007). Support for stabilizing
71 coexistence has largely come from plant and animal communities (Cáceres 1997; Angert *et al.*
72 2009; Yenni *et al.* 2017), while evidence for its role in complex microbial systems is less
73 common (Zhang *et al.* 2010). In microbial communities, which contain a disproportionately large
74 number of extinction-prone rare taxa (Sogin *et al.* 2006; Lynch & Neufeld 2015; Shade *et al.*
75 2018), taxa may vary widely in their stability and long-term contributions to diversity. Although
76 some low-abundance taxa reflect transient dynamics, other rare taxa persist for longer periods of
77 time (Alonso-Sáez *et al.* 2015; Lynch & Neufeld 2015; Newton & Shade 2016). While it is
78 unclear whether the persistence of these rare taxa is aided by niche differences and seed banks,
79 their potential coexistence suggests that NFD may be important for maintaining high levels of
80 biodiversity that are commonly observed in microbial communities.

81 In this study, we tracked bacterioplankton dynamics over time in a north temperate lake
82 using high-resolution molecular data to infer ecological processes that maintain microbial
83 diversity. Bacterial communities in fluctuating aquatic environments often exhibit recurrent,
84 seasonal community patterns (Shade *et al.* 2007; Gilbert *et al.* 2012; Fuhrman *et al.* 2015; Ward

85 *et al.* 2017), but the potential mechanisms that contribute to cyclical dynamics and maintain
86 diversity in nature are poorly resolved. We characterized how persistent (and putatively
87 coexisting) taxa respond to environmental fluctuations and used null models to assess whether
88 stabilizing biotic interactions (e.g., self-limitation, as evidenced by strong NFD) help maintain
89 rare, but metabolically active, taxa in the community (Yenni *et al.* 2017; Rovere & Fox 2019).
90 Specifically, we compared patterns of NFD and population dynamics in the active and total
91 portions of the community (inferred by 16S rRNA transcripts and genes, respectively) to
92 quantify the importance of slow growth or dormancy strategies for the maintenance of diversity.
93 Consistent with predictions from the storage effect, our results provide empirical evidence that
94 stabilizing biotic interactions and seed bank dynamics underlie seasonal community dynamics
95 and play key roles in maintaining bacterial diversity in natural ecosystems.
96

97 **METHODS**

98 *Study site and sampling:* University Lake is a 3.2 ha meso-eutrophic reservoir located in
99 the Indiana University Research and Teaching Preserve, Bloomington, Indiana, USA (39°11' N,
100 86°30' W). The surrounding watershed is dominated by oak, beech, and maple forests. Three
101 streams drain into University Lake, which has an estimated volume of 150,000 m^3 and a
102 maximum depth of 10 m. From April 2013 to September 2015, we took weekly water samples
103 from the surface (0-0.5 m) of the epilimnion for microbial biomass, and measured environmental
104 variables commonly associated with aquatic microbial community dynamics: total phosphorus
105 (TP), total nitrogen (TN), and dissolved organic carbon (DOC). Microbial biomass was filtered
106 on 0.2 µm Supor filters (Pall, Port Washington, NY, USA) and frozen at -80 °C. We quantified
107 TP using the ammonium molybdate method (Wetzel & Likens 2000) and TN with the second

108 derivative method after persulfate digestion (Crumpton *et al.* 1992). DOC was quantified on 0.7
109 µm filtrates using nondispersive infrared (NDIR) detection on a Shimadzu TOC-V (Kyoto,
110 Japan). We also quantified water transparency with a Secchi disk and used a Quanta Hydrolab
111 (OTT, Kempton, Germany) water sonde to measure temperature, conductivity, dissolved oxygen,
112 salinity, and pH of the samples.
113
114 *Bacterial community structure:* We characterized the structure of the bacterial
115 community using high-throughput 16S rRNA sequencing. We extracted total nucleic acids from
116 biomass retained on 0.2 µm filters using the MoBio PowerWater RNA extraction kit and the
117 DNA elution accessory kit. Because sequences obtained from DNA can come from
118 metabolically active or inactive (e.g., slow growing or dormant) individuals, this sample
119 represents the "total" community. In contrast, RNA is a more ephemeral molecule that is
120 essential for synthesizing proteins; therefore, it is often used to characterize the metabolically
121 "active" subset of the community (Molin & Givskov 1999; Steiner *et al.* 2019; Locey *et al.*
122 2020). After extracting nucleic acids, we used DNase (Invitrogen) to remove DNA from the
123 RNA extractions and then synthesized cDNA with SuperScript III First Strand Synthesis kit and
124 random hexamer primers (Invitrogen). To amplify the 16S rRNA gene (DNA) and transcripts
125 (cDNA), we used barcoded V4 primers (515F and 806R) designed for the Illumina MiSeq
126 platform (Caporaso *et al.* 2012). We then purified the PCR products with AMPure XP,
127 quantified DNA concentrations using PicoGreen, and pooled samples at 10 ng per sample. The
128 resulting libraries were sequenced on an Illumina MiSeq at the Indiana University Center for
129 Genomic and Bioinformatics Sequencing Facility using 250 × 250 bp paired end reads (Reagent
130 Kit v2). Paired-end sequences were subsequently processed using the software package mothur

131 (version 1.41.1) (Schloss *et al.* 2009). We assembled contigs, removed low quality sequences
132 (minimum score of 35), aligned sequences to the SILVA Database (version 132) (Quast *et al.*
133 2013), removed chimeras using the VSEARCH algorithm (Rognes *et al.* 2016), and created 97%
134 similar operational taxonomic units (OTUs) using the OptiClust algorithm (Westcott & Schloss
135 2017), and classified sequences with the RDP taxonomy (Cole *et al.* 2009). To account for
136 variation in sequencing depth, subsequent analyses were performed on rarefied abundance data
137 subsampled to the fewest number of reads in the time series (N = 5979 per sample) using R
138 (version 3.6.0) (R Core Team 2018).
139
140 *Differential responses to environment:* To evaluate the first criterion of the storage effect,
141 we looked for signatures of niche partitioning along a suite of environmental variables. First, we
142 performed a principal component analysis (PCA) on Hellinger-transformed abundances to
143 visualize seasonal patterns of compositional trajectories. Next, we identified environmental
144 drivers of community dynamics using redundancy analysis (RDA). We then looked more closely
145 at the persistent, and potentially coexisting, subset of taxa in the community (OTUs present in
146 ≥80% of the DNA-based samples, Table S1). To determine whether environmental fluctuations
147 facilitated temporal niche partitioning, we identified the week of the year when each persistent
148 OTU (n = 82) experienced its maximum average growth rate (see calculations in *Stabilizing*
149 *biotic interactions* below). For each of the persistent OTUs, we compared the environmental
150 conditions at the time of the year when they experienced maximum growth (as an index of
151 optimal conditions along an annual environmental cycle). Specifically, we performed a principal
152 component analysis (PCA) on the environmental variables including temperature, specific
153 conductivity, Secchi depth, pH, TP, TN, and DOC (all standardized to mean = 0, standard

154 deviation = 1). We plotted each time point along the first two PC axes, along with the PC
155 loadings of the environmental variables, and labeled each point with the name of the OTU that
156 exhibited maximum growth at that particular time point (Fig. S1).
157
158 *Seed bank dynamics:* To assess the importance of the next criterion of the storage effect,
159 buffered population growth, we analyzed the temporal role of the seed bank for maintaining
160 bacterial diversity. First, we examined whether the seed bank served as a reservoir of taxonomic
161 diversity by comparing the ratio of total richness to active richness at each time point in the time
162 series, where larger ratios indicate that the total community had higher α-diversity than the active
163 subset of the community. Second, we sought to determine whether seed bank dynamics were
164 more important for the maintenance of rare or common taxa in the community. To do so, we
165 developed a reactivation metric to quantify each OTUs frequency of reactivation from the seed
166 bank. For each OTU, its reactivation score is the number of times an OTU was present (i.e.,
167 detected in the DNA pool) but likely in an inactive (i.e., absent from the RNA pool) state at time
168 point t, yet active (present in the RNA pool) at the subsequent time point $t+1$. This represents a
169 transition from the inactive to active state mediated by slow growth or dormancy based on
170 recovery of sequences in the DNA and RNA pools. Thus, OTUs with higher reactivation scores
171 may be more reliant on the seed bank for long-term persistence in the community. We then
172 analyzed the relationship between the average relative abundance of active OTUs (excluding
173 zeroes) and their reactivation score to determine whether seed banking was more important for
174 maintaining rare taxa than common taxa in the bacterioplankton community.
175

176 *Stabilizing biotic interactions:* In relation to the last criterion of the storage effect, that
177 taxa experience the strongest self-limitation during favorable environments, we examined
178 patterns of negative frequency dependence in the community. In particular, we examined (1)
179 whether growth exhibited negative frequency dependence overall, which is indicative of
180 stabilization in the community, and (2) whether rare taxa experienced stronger negative
181 frequency dependence than common taxa (Yenni *et al.* 2012, 2017; Rovere & Fox 2019). Such
182 patterns of NFD shed light on the storage effect for three reasons. First, taxa are more likely to be
183 common in the community when they are environmentally favored relative to other taxa in the
184 community. Second, taxa that experience strong intraspecific limitation during environmentally
185 favorable periods would grow faster when rare than when common, thereby generating NFD.
186 Third, differences among taxa in the strength of self-limitation during favored growth periods
187 would lead to different average relative abundances in the community, implying that rare yet
188 persistent taxa may by strongly stabilized (Yenni *et al.* 2012, 2017; Rovere & Fox 2019).
189 However, metabolically inactive individuals are unlikely to engage in the biotic interactions that
190 generate NFD. Therefore, we focused on NFD in the metabolically active (i.e., RNA-based)
191 portion of the community comprised of the persistent taxa (Table S1).
192 We then calculated NFD for each OTU by comparing rates of change in relative
193 abundance between weekly samples. We inferred the strength of NFD for a given OTU as the
194 magnitude of the negative slope of the relationship between an OTU's relative abundance and its
195 per capita growth rate at each time step (t) across the time series. We calculated the relative
196 abundance ($x_{t,s}$), of each OTU (s) as its abundance ($N_{t,s}$) in the community of s OTUs relative
197 to the total abundance of all s OTUs ($\sum_s N_{t,s}$) at a given time step (t), such that $x_{t,s} = \frac{N_{t,s}}{\sum_s N_{t,s}}$.
198 From this, we then calculated the natural log of the per capita growth rate of each OTU as $y_{t,s} =$

199 $\log_e\left(\frac{N_{t+1,s}}{N_{t,s}}\right)$. To estimate the strength of negative frequency dependence for each OTU, we fit

200 simple linear regressions ($y_s = \beta_{0,s} + \beta_{1,s}x_s + \epsilon_s$), where the equilibrium frequency of an OTU

201 (f) is the x-intercept, $f = -\frac{\beta_{0,s}}{\beta_{1,s}}$, and the degree of frequency dependence (NFD) is the slope

202 $NFD = \beta_{1,s}$. In the end, f describes whether an OTU is common or rare, and negative slopes

203 with greater magnitudes indicate stronger negative frequency dependence.

204

205 *Stabilization of rare bacterial taxa:* We used null models to infer whether rare taxa

206 exhibited stronger NFD than expected by chance. Stabilization of rare taxa would be supported if

207 OTUs with lower equilibrium frequencies (smaller values of f) had more negative slopes (larger

208 $|\beta_{1,s}|$) indicating stronger self-limitation. We inferred the overall relationship between rarity and

209 strength of NFD from the covariance between the $\log(NFD)$ and $\log(f)$: a more negative

210 covariance would indicate that rarer taxa were more strongly stabilized than common taxa. To

211 account for the fact that the expectation of this covariance is already negative, and to control for

212 spurious statistical correlations in the temporal data due to other factors, we implemented a null

213 model approach (Yenni *et al.* 2017; Rovere & Fox 2019). We shuffled the abundances of each

214 OTU independently, recalculated relative abundances and per capita growth rates, estimated

215 equilibrium frequencies (fs) and negative frequency dependences (NFDs), and calculated the

216 covariance, repeating this procedure 5000 times to generate a null distribution of covariance

217 values ($COV[\log(f), \log(NFD)]$) (Yenni *et al.* 2017). We then compared our observed

218 covariance with the null distribution to infer the strength of asymmetry in NFD (i.e., the degree

219 to which rare OTUs experience disproportionately stronger self-limitation than common OTUs).

220 We quantified divergence from null distributions using standardized effect sizes (SES = mean

221 observed covariance / standard deviation of covariances in the null distribution) and the ratio of
222 observed covariance to the average covariance of the null distribution (Yenni *et al.* 2017). More
223 negative SES values and larger ratios would indicate greater deviations from the null expectation
224 of equal NFD across taxa. We inferred the degree of statistical significance by calculating a p-
225 value as the proportion of null covariance values less than or equal to our observed covariance.
226

227 **RESULTS**

228 *Differential responses to environment:* Bacterial community dynamics were related to
229 environmental variability, with different taxa favored at different times of the year. During the
230 summer months, the community followed a recurrent successional trajectory (Fig. 1A). This
231 trajectory was strongly aligned with seasonal trends in temperature (Fig. 1B). Across longer time
232 scales, inter-annual variation in dissolved oxygen and pH was associated with compositional
233 differences in the active bacterial community during winter months. Within an annual cycle, the
234 persistent OTUs (n = 82) demonstrated temporal partitioning in their maximal growth rates in the
235 active portion of the community (Fig. 2, Table S1), corresponding to different environmental
236 conditions (Fig. S1).
237

238 *Seed bank dynamics:* Our data suggest seed banks of dormant or slow growing
239 individuals contribute to the maintenance of diversity. Over the course of our study, total
240 richness ranged from 1.2–2.0 times higher than the richness of the active portion of the
241 community (Fig. 3). Furthermore, this discrepancy between total and active richness exhibited
242 seasonality, demonstrating a time-varying role for the bacterial seed bank. In particular, the seed
243 bank played a weaker role (i.e., active and total richness were more similar in magnitude) during

244 the summer, while proportionally higher diversity was found in the seed bank over winter, when
245 growing conditions may be less optimal (Fig. 3). In addition, the taxa that exhibited more
246 reactivations from the seed bank were also the taxa that were, on average, consistently rare when
247 active in the community (Fig. 4). In contrast, common taxa exhibited fewer transitions between
248 active and inactive states in the community. Thus, our findings suggest that rare taxa in the
249 community rely on life-history strategies, such as slow growth or dormancy, to avoid local
250 extinction.
251
252 *Stabilizing biotic interactions:* Persistent taxa exhibited stabilizing NFD, which varied in
253 strength depending on each taxon's mean relative abundance in the community (Fig. S2). In
254 particular, NFD was significantly stronger for rare taxa than common taxa, but only in the active
255 portion of the community ($p = 0.0002$; SES = -4.03, covariance ratio = 1.08), not in the total
256 community ($p = 0.221$; SES = -0.777, covariance ratio = 1.01) (Fig. 5). The p-values reflect the
257 rank of observed NFD compared with null simulations, while SES values take into account the
258 variance in the null distribution. In other words, the total community showed nearly the same
259 degree of stabilization ($|SES| < 2$, covariance ratio ~ 1) as the null communities.
260
261
262 **DISCUSSION**
263 Our findings from a multi-year survey support the view that biodiversity was maintained
264 by stabilizing mechanisms, including niche differentiation and seed bank dynamics, that
265 generated negative frequency dependence (NFD) in a natural bacterioplankton community. High
266 resolution sampling revealed recurrent seasonality in community dynamics (Fig. 1), driven by

267 taxon-specific responses to annual environmental fluctuations (Fig. 2, Fig. S2). Our results also
268 showed that the maintenance of diversity may be enhanced by life-history strategies, such as
269 slow growth or dormancy, that buffered rare taxa from local extinction during environmentally
270 unfavorable periods (e.g., winter) and facilitated reestablishment when conditions improved (Fig.
271 3-4). These apparent niche differences and seed bank dynamics contributed to stabilizing biotic
272 interactions (e.g., stronger intra- than interspecific limitation) among rare, but metabolically
273 active, taxa in the community (Fig. 5).
274
275 *Negative frequency dependence in microbial communities:* We found evidence for
276 stabilization through negative frequency dependence (NFD). While documented in some plant
277 and animal assemblages (Harpole & Suding 2007; Yenni *et al.* 2017; Rovere & Fox 2019),
278 observations of NFD in complex microbial communities are uncommon. In particular, our study
279 revealed disproportionately strong NFD for rare taxa, offering an explanation for why some taxa
280 appear to stably persist at low relative abundances in nature (Alonso-Sáez *et al.* 2015; Lindh *et*
281 *al.* 2015), potentially as members of the "rare biosphere" (Sogin *et al.* 2006; Lynch & Neufeld
282 2015; Shade *et al.* 2018). Our approach also allowed us to identify stabilizing mechanisms
283 operating among metabolically active rare taxa that were not detectable from the dynamics of the
284 total bacterial community (Fig. 5). Ignoring this metabolic heterogeneity can obscure inferences
285 of underlying ecological processes (Wisnoski *et al.* 2020), and would have gone otherwise
286 undetected in this study as well.
287 Asymmetric NFD is also important for the coexistence of rare plant and animal species,
288 but the magnitude of this effect varies across taxonomic groups (Yenni *et al.* 2017; Rovere &
289 Fox 2019). For example, NFD is less asymmetric for herpetofauna than plant or mammal

290 communities (Yenni *et al.* 2017), possibly due to higher evenness (Rovere & Fox 2019).
291 Compared to macro-organismal systems, the degree of NFD asymmetry in our highly uneven
292 bacterial community was moderate (SES = -4.03, covariance ratio = 1.08), suggesting that
293 coexistence among rare taxa may be weak, or that additional factors not captured by this metric
294 are important for maintaining diversity in this system. However, our comparison between the
295 metabolically active and total portions of the bacterioplankton community provides critical
296 evidence that active bacteria mediate the biotic interactions that generate asymmetry in NFD and
297 allow rare taxa to persist. Consistent with prior work showing that rare taxa may be
298 disproportionately active in freshwater bacterial communities (Jones & Lennon 2010), our study
299 demonstrates that rare, metabolically active bacteria may also be critical for the long-term
300 maintenance of bacterial diversity.
301
302 *Storage effect in microbial communities:* Temporal niche partitioning mediated by seed
303 bank dynamics may explain the maintenance of diversity in fluctuating environments. Evidence
304 for coexistence via the storage effect comes largely from communities of desert annuals (Pake &
305 Venable 1996; Angert *et al.* 2009), grasslands (Adler *et al.* 2006), tropical trees (Usinowicz *et al.*
306 2012), zooplankton (Cáceres 1997), and marine fish (Secor 2007). In most bacterial studies, the
307 role of seed banks for coexistence has been inferred from short-term observations, but here we
308 provide temporal evidence that bacterial seed banks may be important for community dynamics
309 and the maintenance of diversity. In the temperate climate of our study lake, different taxa
310 showed maximum growth rates at different times of the year, coinciding with seasonal transitions
311 in environmental conditions (Fig. 2), and contrasting active and total community dynamics
312 suggested buffered population dynamics (Figs. 3-5). In addition, species showed NFD in their

growth such that they experienced greater self-limitation (consistent with stronger intraspecific than interspecific competition) when they were more common in the active community (and were thus more likely favored by the environment) (Fig. 5, Fig. S2). While these conditions are not definitive proof of a storage effect acting in our study system, they are consistent with the criteria needed for a storage effect to contribute to the long-term maintenance of bacterial diversity in the lake.

Seed banks may also have more general implications for bacterial community dynamics. The persistence of taxa with temporal niche differences may contribute to the repeatability of summer community dynamics in the active portion of the community (Hellweger *et al.* 2008) by favoring overwinter survival (Fig. 3). For example, the seasonality of the seed bank indicates that diversity stored in the seed bank was maximized when environmental conditions (e.g., water temperature, resource/consumer densities) were least favorable for bacterial growth (Neuenschwander *et al.* 2015). This pattern is consistent with the presence of a dormant seed bank that buffers individuals from harsh conditions. In addition, transitions from inactive to active metabolic states were more frequently detected among taxa that were, on average, rare when active in the community. Analogous to the methodological challenges of finding and identifying dormant individuals in non-microbial seed banks (e.g., plants), RNA detection limits may affect the classification of metabolically active bacteria. Nevertheless, our reactivation metric should capture rapid shifts in the metabolically active portion of the community. Overall, these patterns suggest that recurrent environmental cues regulate active community dynamics by favoring different taxa at different times of the year, and that seed banks are important for maintaining these seasonal community trajectories at multi-annual timescales.

336 *Future directions and conclusions:* Our study provides empirical evidence supporting the
337 theory that biotic mechanisms stabilize bacterial communities and maintain diversity in nature.
338 While our results indicate differences in the diversity, dynamics, and stabilization between active
339 and total subsets of the community, an ultimate goal is to tighten the mechanistic links between
340 rates of ribosomal RNA transcription and *in situ* growth rates for individual taxa (Newton &
341 Shade 2016; Papp *et al.* 2018) or through other techniques that involve the physical sorting of
342 cells based on metabolic activity prior to sequencing (Couradeau *et al.* 2019; Reichart *et al.*
343 2020). It may also be important to consider the dispersal of terrestrial bacteria into aquatic
344 ecosystems (Crump *et al.* 2012). However, previous work in our study system revealed that most
345 terrestrial bacteria were metabolically inactive, most likely reflecting the abrupt environmental
346 transitions that accompany cross-ecosystem dispersal (Wisnoski *et al.* 2020). Thus, the lack of
347 asymmetric NFD we observed in the total community could also arise in part from allochthonous
348 inputs of inactive bacteria that decouple population growth of each OTU from its relative
349 abundance in the community. However, by focusing on the dynamics of metabolically active
350 bacteria, our approach was able to uncover the presence of stabilizing biotic interactions that
351 may have otherwise been obscured by metabolic heterogeneity in the total community.
352 In conclusion, we show that stabilizing biotic interactions and the ability to engage in
353 dormancy or slow growth strategies play important roles in maintaining microbial diversity in a
354 natural ecosystem over a multi-year time scale. Our results demonstrate the mechanisms at the
355 community scale that preserve Earth's vast microbial diversity, building on other explanations
356 that emphasize the importance of metabolic diversity (Sala *et al.* 2008), capacity for rapid growth
357 (Shade *et al.* 2014), and spatial scale (Vos *et al.* 2013). In particular, strong NFD offers a new
358 explanation for why the majority of bacterial taxa persist at low average relative abundances in

nature (Lynch & Neufeld 2015). Furthermore, our work builds on inferences about the roles of microbial dormancy (and other persistence strategies) obtained from shorter time scales, and provides temporal evidence that dormancy is an important buffer against local extinction over longer time scales. More generally, our work demonstrates the importance of stabilization in microbial systems, offering new insight into the long-term maintenance of microbial diversity.

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523 **FIGURE LEGENDS**

524 **Figure 1.** Seasonal dynamics of the active bacterial community in University Lake. (A) The
525 compositional trajectory of the active community (determined by high-throughput sequencing of
526 16S rRNA transcripts) shows strong seasonality, but the community remains relatively static
527 over winter. The first two axes of the principal component analysis (PCA) depict summer/winter
528 differences (PC1) along the major axis, and slight inter-annual differences in winter composition
529 (cool colors) along the minor axis (PC2). The summer successional trajectory (warmer colors) is
530 highly repeatable across years. (B) Constrained ordination using redundancy analysis (RDA)
531 shows the environmental drivers of community structure, along with strong correlates of
532 individual taxa in the community. This analysis reveals that differences in pH and dissolved
533 oxygen (DO) explain variation in winter composition among years.

534

535 **Figure 2.** Temporal partitioning of maximum growth rate among persistent bacterial taxa in
536 University Lake. Lines represent the mean daily growth rate for each taxon over the time series.
537 Points indicate the maximum growth observed for each bacterial taxon (OTU). Overall, the 82
538 persistent OTUs have maximum growth rates at different seasons of the year. Points are color-
539 coded such that warmer colors correspond to spring and summer months and cooler colors
540 correspond to winter months. Colored lined trace out the growth dynamics of three individual
541 taxa with different environmental responses (blue = OTU 1, Betaproteobacteria; yellow = OTU
542 17, Actinobacteria; mauve = OTU 18, Gammaproteobacteria). More taxonomic details can be
543 found in Table S1.

544

545 **Figure 3.** Seasonal importance of the seed bank for bacterial diversity in University Lake.
546 Richness was much higher in the total community, relative to the active community, during the
547 fall and winter months. The active and total communities converged over the summer, indicated
548 by values on the y-axis closer to 1. Warmer colors correspond to spring and summer months,
549 while cooler colors correspond to winter months. Shaded regions correspond to the fall and
550 winter months (October through March).
551
552 **Figure 4.** Rare taxa showed more seed bank transitions than common taxa. For the 82 persistent
553 taxa identified over the time series, OTUs that were (on average) rare in the active portion of the
554 community had a higher number of reactivations from the seed bank, while more common taxa
555 had fewer reactivations. The fit was performed via locally estimated scatterplot smoothing
556 (LOESS).
557
558 **Figure 5.** Asymmetric negative frequency dependence (NFD) for persistent bacterial taxa (n =
559 82) was only detected in the active portion of the community. The degree of asymmetry in NFD
560 is determined by the covariance between the equilibrium frequency of each OTU and its strength
561 of NFD; negative covariance indicates that rarer taxa exhibit stronger NFD. Compared with
562 expected covariances from a null distribution, the standardized effect size (SES) of the observed
563 covariance in the active portion of the community was -4.03, while the SES of the total
564 community was -0.77. The overall strength of NFD (observed NFD / mean NFD) was 1.08 in the
565 active portion and 1.01 in the total community. The metabolic state on the y-axis indicates
566 whether the NFD comparison is for the active portion of the community (inferred from 16S
567 rRNA transcripts) or the total portion (inferred from 16S rRNA genes, i.e., DNA).

FIGURES

Figure 1

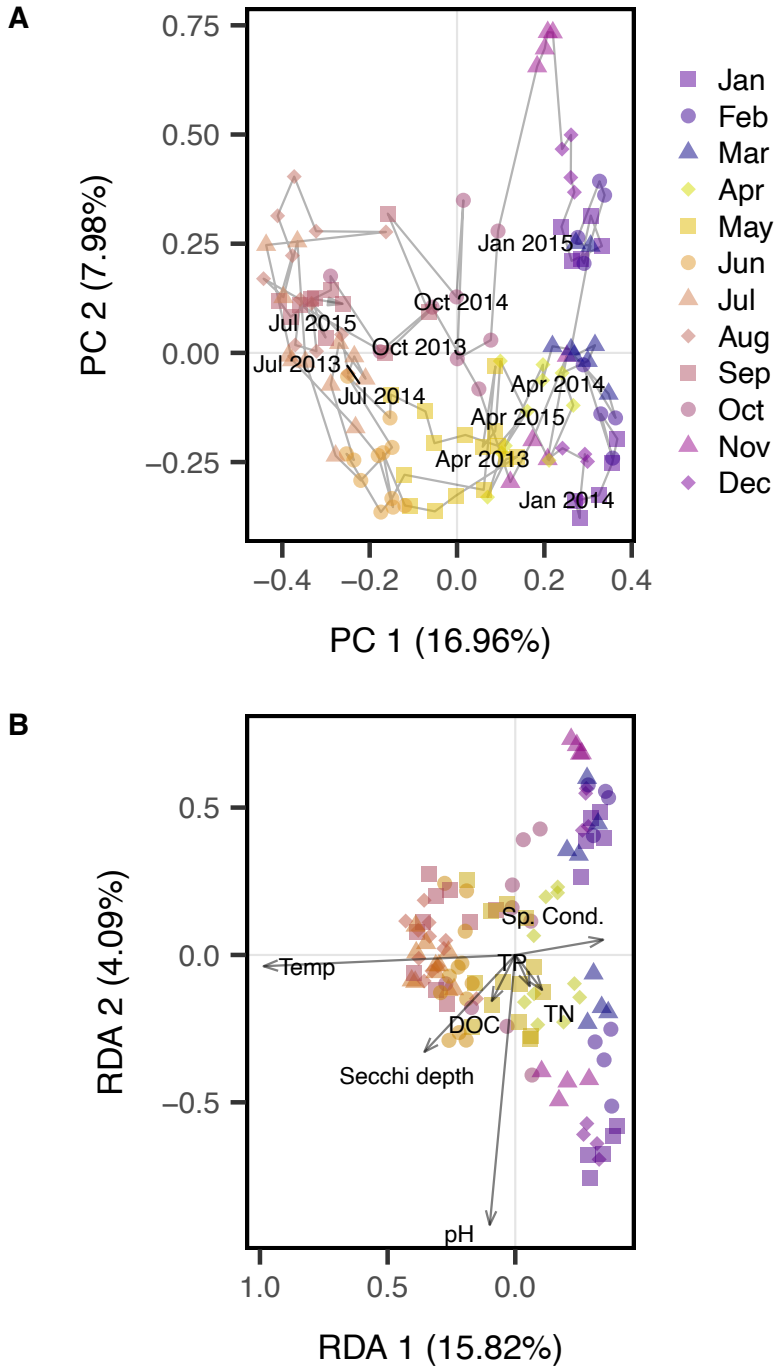


Figure 2.

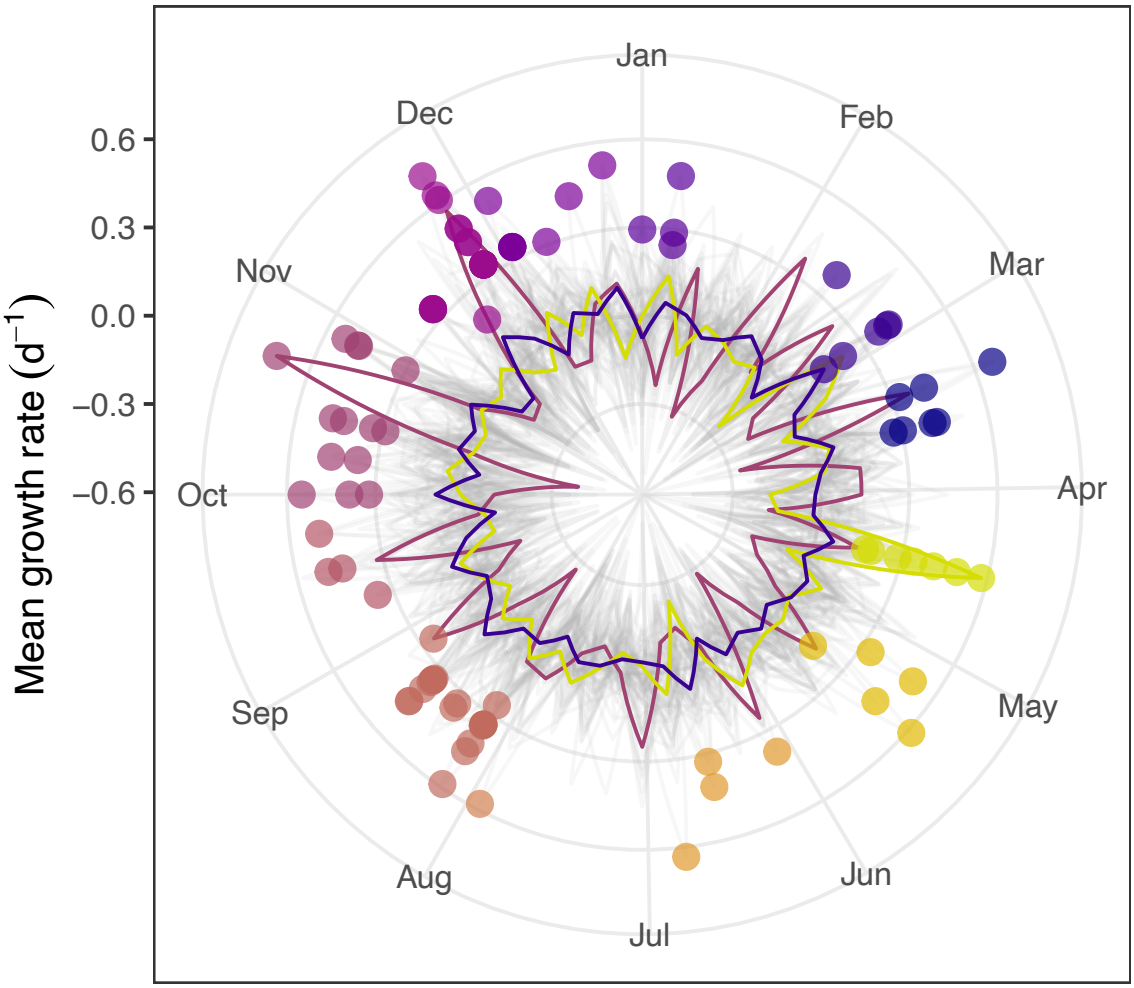

Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
0.6
0.3
0.0
−0.3
−0.6
Mean growth rate (d^{-1})

Figure 3.

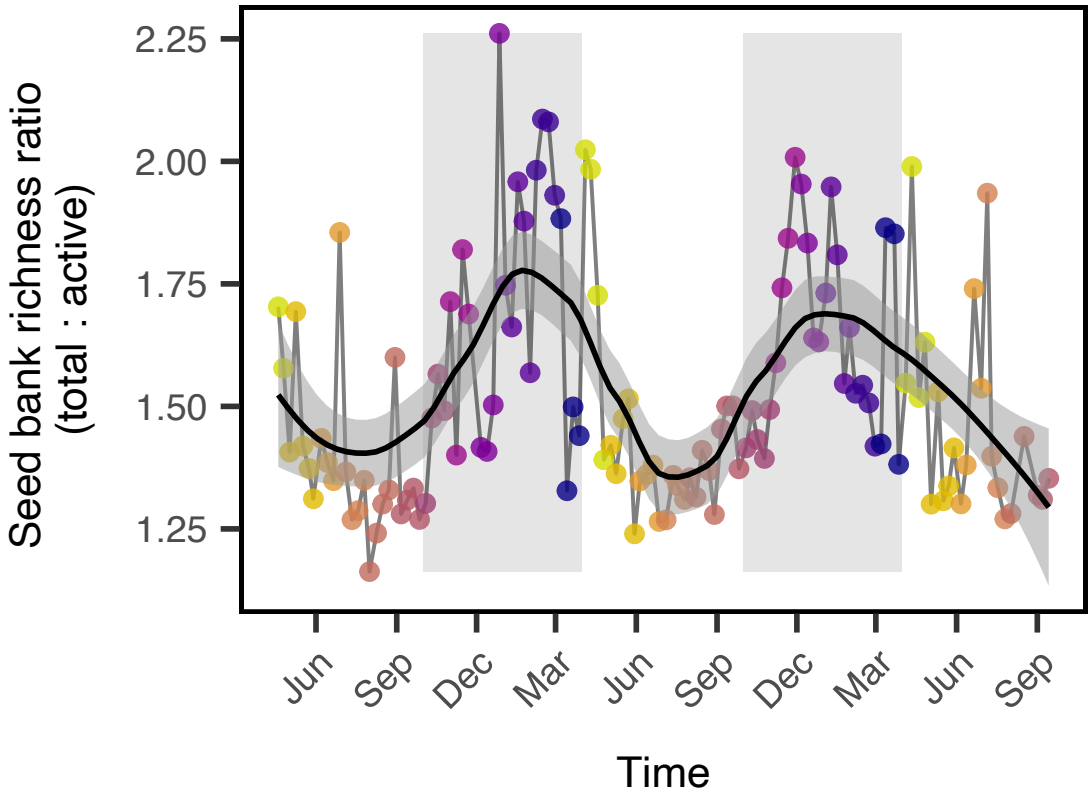
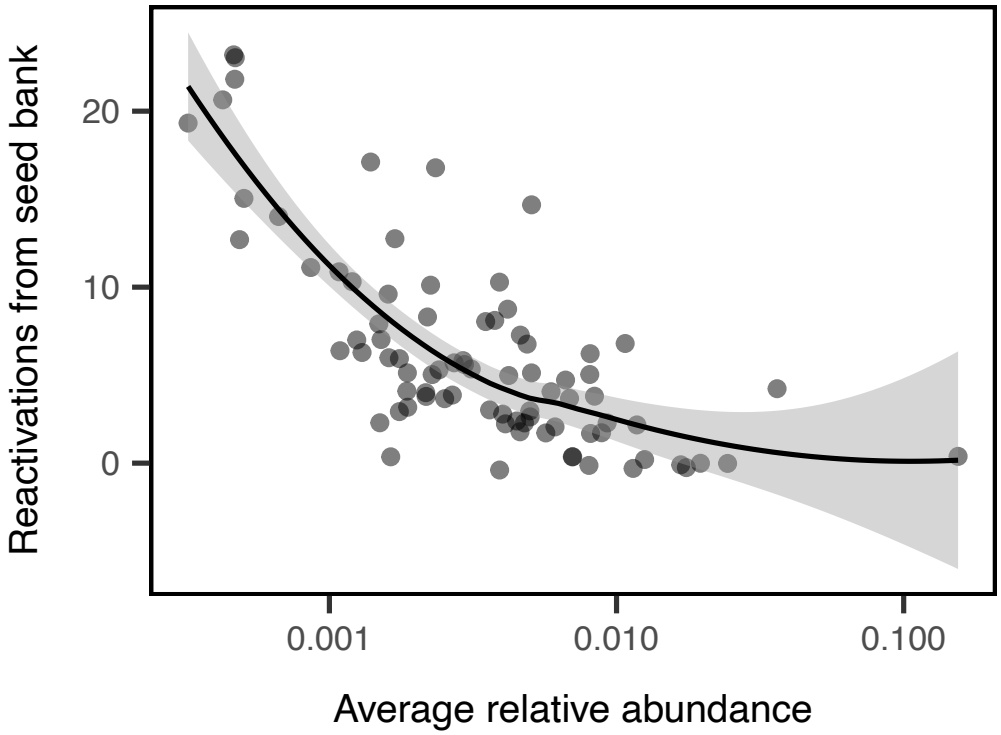
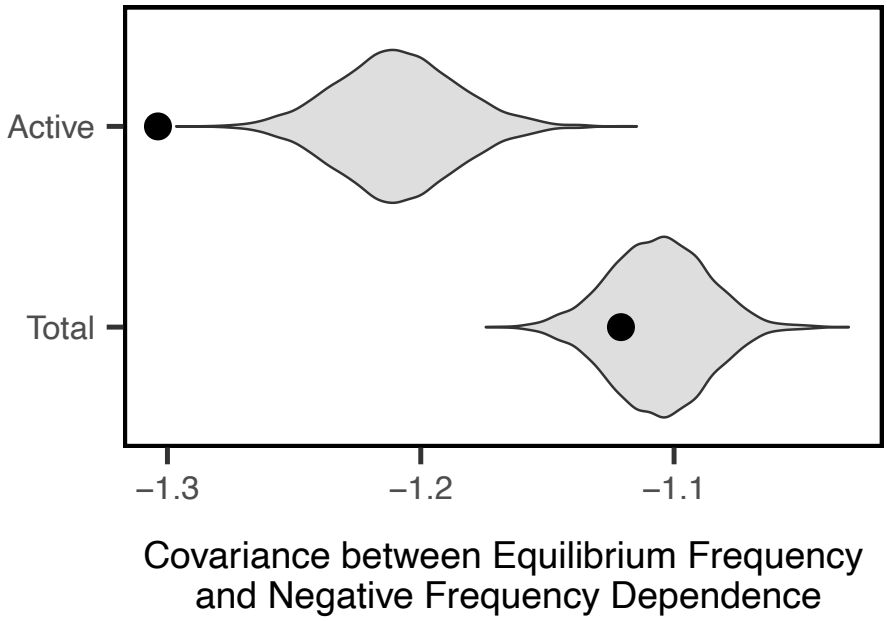

Seed bank richness ratio
(total : active)
2.25
2.00
1.75
1.50
1.25
Jun
Sep
Dec
Mar
Jun
Sep
Dec
Mar
Jun
Sep
Time

Figure 4.


Reactivations from seed bank
20
10
0
0.001
0.010
0.100
Average relative abundance

584

585 Figure 5.



586

587

588

589 **SUPPLEMENTAL INFORMATION**

590 **Table S1**. Operational taxonomic units (OTUs) that were classified as persistent in the

591 bacterioplankton community based on being detected in ≥80% of the total (i.e., DNA)

592 community samples. The table is sorted by Julian date of max growth.

OTU	Class	Max growth rate (d^{-1})	Date of max growth
Otu00034	Alphaproteobacteria	0.3426	2014-01-17
Otu00045	Betaproteobacteria	0.8527	2014-01-03
Otu00196	Actinobacteria	0.5873	2015-01-09
Otu00019	Cytophagia	0.7346	2014-02-14
Otu00039	Betaproteobacteria	0.7167	2014-02-14
Otu00102	Betaproteobacteria	0.6729	2015-02-28
Otu00105	Alphaproteobacteria	0.8666	2014-02-28
Otu00065	Sphingobacteriia	0.8563	2014-03-21
Otu00292	Alphaproteobacteria	0.6851	2014-03-07
Otu00006	Sphingobacteriia	0.3643	2013-04-25
Otu00012	Betaproteobacteria	0.8784	2014-04-18
Otu00014	Actinobacteria	0.4906	2015-04-26
Otu00016	Actinobacteria	0.7836	2014-04-18
Otu00017	Actinobacteria	1.0200	2015-04-04
Otu00021	Gammaproteobacteria	1.0449	2014-04-18
Otu00033	Alphaproteobacteria	0.5306	2014-04-25
Otu00048	Verrucomicrobiae	0.7426	2015-04-11

Otu00049	Actinobacteria	0.6090	2014-04-04
Otu00055	Flavobacteriia	0.7950	2014-04-18
Otu00058	Armatimonadia	0.8153	2015-04-11
Otu00148	Bacteria sp.	0.7646	2013-04-25
Otu00172	Gammaproteobacteria	0.6729	2015-04-11
Otu00219	Betaproteobacteria	0.7503	2014-04-18
Otu00002	Actinobacteria	0.3190	2015-05-03
Otu00008	Actinobacteria	0.3637	2013-05-09
Otu00022	Opitutae	0.8003	2015-05-03
Otu00031	Cytophagia	0.6593	2014-05-09
Otu00051	Flavobacteriia	0.9187	2013-05-09
Otu00062	Flavobacteriia	0.7836	2015-05-23
Otu00064	Alphaproteobacteria	0.6964	2013-05-29
Otu00113	Bacteroidetes sp.	0.7950	2013-05-09
Otu00116	Betaproteobacteria	0.8373	2014-05-09
Otu00151	Betaproteobacteria	0.7259	2013-05-17
Otu00183	Bacteria sp.	0.6090	2015-05-03
Otu00200	Bacteria sp.	0.6851	2015-05-23
Otu00083	Flavobacteriia	1.0031	2015-06-06
Otu00087	Betaproteobacteria	0.5873	2014-06-05
Otu00098	Betaproteobacteria	0.8104	2013-06-14
Otu00123	Sphingobacteriia	0.7259	2014-06-20
Otu00194	Deltaproteobacteria	0.9277	2014-06-13

Otu00294	Alphaproteobacteria	0.6964	2013-06-21
Otu00004	Actinobacteria	0.3500	2015-07-11
Otu00009	Gammaproteobacteria	1.3329	2013-07-26
Otu00011	Betaproteobacteria	0.9841	2015-07-18
Otu00192	Bacteria sp.	0.4350	2013-07-26
Otu00195	Actinobacteria	0.6277	2014-07-18
Otu00020	Betaproteobacteria	0.4150	2013-08-01
Otu00026	Betaproteobacteria	0.6277	2013-08-01
Otu00029	Actinobacteria	0.6593	2013-08-23
Otu00036	Alphaproteobacteria	0.7576	2013-08-16
Otu00037	Actinobacteria	0.7646	2014-08-29
Otu00052	Alphaproteobacteria	0.6444	2013-08-09
Otu00076	Actinobacteria	0.6444	2014-08-08
Otu00112	Alphaproteobacteria	0.5873	2014-08-23
Otu00226	Opitutae	0.6729	2015-08-02
Otu00250	Actinobacteria	0.5306	2013-08-23
Otu00010	Proteobacteria sp.	0.6277	2014-09-26
Otu00024	Bacteroidetes sp.	0.7259	2014-09-19
Otu00056	Cytophagia	0.7711	2013-09-06
Otu00067	Betaproteobacteria	0.5306	2015-09-02
Otu00177	Proteobacteria sp.	0.5306	2013-09-20
Otu00005	Sphingobacteriia	0.5294	2014-10-04
Otu00015	Actinobacteria	0.6277	2014-10-17

Otu00018	Gammaproteobacteria	0.9516	2014-10-11
Otu00060	Betaproteobacteria	1.0031	2013-10-25
Otu00066	Betaproteobacteria	0.8153	2013-10-25
Otu00082	Bacteroidetes sp.	0.8003	2014-10-04
Otu00154	Alphaproteobacteria	0.7070	2014-10-04
Otu00158	Gammaproteobacteria	0.9141	2013-10-04
Otu00001	Betaproteobacteria	0.2860	2013-11-15
Otu00007	Betaproteobacteria	0.4577	2013-11-15
Otu00038	Actinobacteria	0.7346	2014-11-29
Otu00047	Betaproteobacteria	0.6729	2013-11-15
Otu00073	Betaproteobacteria	0.4906	2013-11-22
Otu00077	Flavobacteriia	0.7774	2013-11-15
Otu00109	Actinobacteria	0.6729	2013-11-15
Otu00118	Actinobacteria	0.6090	2013-11-22
Otu00129	Alphaproteobacteria	0.4906	2013-11-15
Otu00198	Betaproteobacteria	0.9210	2013-11-15
Otu00208	Betaproteobacteria	0.6964	2013-11-22
Otu00217	Proteobacteria sp.	0.7167	2013-11-22

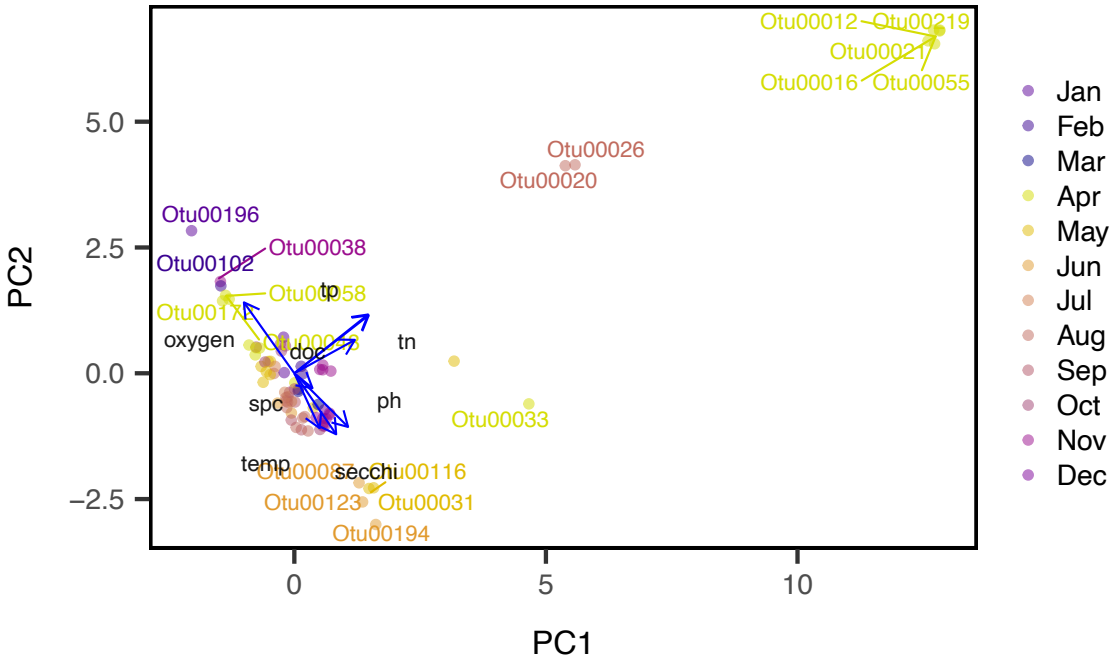
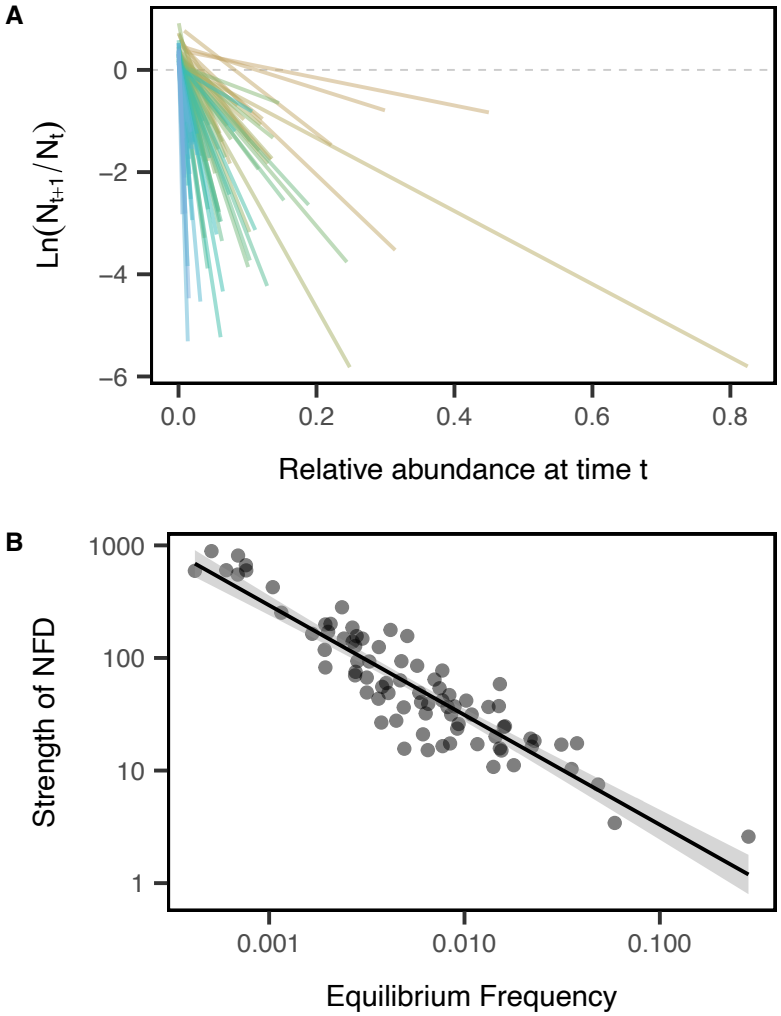


Figure S1. Temporal partitioning of maximum growth rates corresponds to environmental conditions. Here, we depict a principal component analysis of the environmental conditions at each date. OTU labels indicate the individual taxa that exhibit maximum growth at the corresponding time of the year. Vectors indicate the loadings of environmental variables along the two PC axes. Warmer colors correspond to spring and summer months, cooler colors correspond to winter months.



603

604 **Figure S2.** Negative frequency dependence (NFD) in the active portion of the community for the
605 82 persistent bacterial taxa. (A) Relationship between the rate of change of an OTU and its
606 relative abundance. Depicted in this graph are simple linear regression fits for the 82 taxa
607 individually, data points not shown to reduce clutter. Negative relationships indicate NFD
608 growth and variation in slopes indicates variation in the strength of NFD. (B) Rare taxa (lower
609 equilibrium frequencies) exhibit stronger NFD, while common taxa (higher equilibrium
610 frequency) have weaker NFD.

611